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The collection of high-quality sediment samples recovered from the seafloor is prerequisite to initiating laboratory investigations of sedimentological, microstructural, or geotechnical properties. These types of studies require virtually undisturbed material. Likewise, microfabric studies require the utmost care in the handling of samples and in the preparation of subsamples for electron microscopic observations. The literature is replete with discussions and techniques concerning submarine sediment coring and sample recovery in general. Richards (1962) presented a thorough review of the various types of coring devices and the significance of sample disturbance. In general, large-diameter thin-wall sampling devices provide high-quality sediment cores (Richards and Keller, 1961; Lambert and Merrill, 1979). High-quality sediment core samples now can be recovered from deep core drilling using specially designed sampling tools (DSDP, Vols. 64 and 68). Analyses and evaluation of sample quality were discussed by Bennett (1976) and Bennett et al. (1977). Discussion of the numerous sampling techniques is beyond the scope of this chapter and the interested reader is referred to the above references for details.

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CHAPTER 35

Techniques for the Preparation of Submarine Sediments for Electron Microscopy

Roy J. Baerwald, Patti J. Burkett, and Richard H. Bennett

Introduction

The collection of high-quality sediment samples recovered from the sea floor is prerequisite to initiating laboratory investigations of sedimentological, microstructural, or geotechnical properties. These types of studies require virtually undisturbed material. Likewise, microfabric studies require the utmost care in the handling of samples and in the preparation of subsamples for electron microscopic observations. The literature is replete with discussions and techniques concerning submarine sediment coring and sample recovery in general. Richards (1962) presented a thorough review of the various types of coring devices and the significance of sample disturbance. In general, large-diameter thin-wall sampling devices provide high-quality sediment cores (Richards and Keller, 1961; Lambert and Merrill, 1979). High-quality sediment core samples now can be recovered from deep core drilling using specially designed sampling tools (DSDP, Vols. 64 and 68). Analyses and evaluation of sample quality were discussed by Bennett (1976) and Bennett et al. (1977). Discussion of the numerous sampling techniques is beyond the scope of this chapter and the interested reader is referred to the above references for details.

The purpose of this chapter is to present proven techniques for the preparation of sediment subsamples for conventional electron microscopy (EM) observations and study. Analytical procedures such as energy dispersive spectroscopy (EDS), wavelength dispersive spectroscopy (WDS), electron energy loss spectroscopy (EELS), and back-scattered electron analysis of polished specimens (Smart and Tovey, 1982) were considered beyond the scope of this chapter. Although many conventional EM techniques can be found in numerous technique manuals for the biologist, few exist for the geologist.

The critical element in the tedious process of sediment sample preparation for EM is to maintain and ensure microfabric integrity. The microfabric is defined as the orientation and arrangement or spatial distribution of the solid particles and their particle-to-particle relationships (Bennett, 1976; Bennett et al., 1977). The microfabric is intimately linked and related to the physicochemistry of the sedimentary material and the terms microfabric and physicochemistry are called, inclusively, the microstructure (Bennett et al., 1977; this volume). Because of the nature of the questions being addressed by scientists and engineers regarding the complex processes and mechanisms that drive the development of sediment microfabric, techniques and instrumentation must be continually pushed to their limits. In addition, new techniques must be developed to enlighten our perspectives and comprehension of the interactive environmental processes that produce the observed features. This chapter presents tested and proven techniques that may help to standardize future studies of microfabric and provide a common base for meaningful comparisons of data and observations among researchers. Some developing technologies and future requirements are discussed in a later section.

Techniques for Clay Sediment Preparation for TEM

Submarine sediment samples are comprised of organic material and assemblages of minerals, including clay, carbonates, and silica and many other rock-forming minerals. The intrinsic physical nature of the sediment types vastly differs, and is dependent on these various minerals comprising the sediment fabric that ultimately governs its physical properties and behavior under static and dynamic stresses. Cohesiveness of the sediment can

vary greatly from weak to noncohesive as found in quartz sands and aragonitic and carbonate sediments (Bennett et al., 1990). Highly cohesive sediments are exemplified by smectite-rich Mississippi delta clays (Bennett, 1976) and Pacific red clays (Bryant and Bennett, 1988; Burkett et al., this volume). Knowledge of the mineralogy and intrinsic strength of the material is essential when determining the best technique for preparing a particular sediment to ensure integrity of the microstructure during the many steps of sample preparation.

A variety of methods for dehydration, infiltration, and embedding and cleaning of sediment soil samples for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have been described historically by Pusch (1966, 1967, 1968), Smart (1967a,b), Bowles (1968a,b), O'Brien (1971), Foster and De (1971), Hulbert and Bennett (1975), and Smart and Tovey (1982). Conventional methods of sample preparation for biological specimens employ "liquid dehydration" procedures with a graded series of fluids such as ethanol or acetone followed by a graded series of propylene oxide and epoxy during infiltration (Pease, 1964; Sjostrand, 1967; Hayat, 1970; Hayat and Zirkin, 1973). Often these conventional methods for biological sample preparation are not advantageous for marine clay sediment, characteristically having low coefficients of permeability ($k = 10^{-6}$–10^{-9} cm/sec) that require long dehydration and infiltration times because of the relatively high viscosities of the epoxy resins. Several specific methods are described in detail in this report. However, it is important to try several techniques simultaneously in the early stages until one method is found to be best for the particular sediment being studied. Bennett (1976) and Bennett et al. (1977) first described application of critical point drying (details of CPD method will be described later) and embedding techniques for submarine clay samples. Computer digitizing of TEM micrographs from samples that were critical point dried demonstrated no change in void ratio (ratio volume of the voids to the volume of the solids) indicating no disturbance of the microfabric (Bennett, 1976). This method has been preferred in subsequent studies (Bennett et al., 1977; Chiou, 1980; Chiou et al., 1983; Bennett and Hulbert, 1986; Burkett, 1987; Burkett et al., this volume). Figure 35.1 is a flow chart of techniques recommended for clay sediment sample preparation used in microfabric analysis. The initial six steps are identical for TEM and SEM, but are markedly different after critical point drying.

Procedures

Step 1. Using a small-diameter wire knife, rectangular-shaped subsamples from sediment cores are slowly cut, immersed in absolute ethanol (ethyl alcohol), and sealed to prevent dehydration. Care must be taken to minimize sampling disturbance during cutting. The rectangular shape of the sample orients the sample with respect to the bedding plane without etching or marking any of the surfaces that could degrade. The center of the sample is the highest quality region for the subsample because it is protected from disturbances such as compression or microshearing or from drag along the core wall. The subsamples should be trimmed to approximately 4 × 7 mm to ensure a proper fit later into BEEM capsules (Fig. 35.2b,c). Sometimes a smaller sample is needed if the sediment resists resin penetration, as do argillitic samples or shales; but, in general, small samples may be more susceptible to disintegration. Since visual recognition of samples is impossible after processing, careful labeling with a pencil, insoluble ink, on small paper labels is essential. Subsample orientation should be maintained to ensure that sectioning or trimming for SEM can be performed in a direction normal to bedding planes for standard observation procedures. Some studies may require precision and control for other directions of observation.

Step 2. The alcohol is carefully exchanged by pipetting or decanting and replacing with fresh alcohol stepwise until all the water is removed. To test for complete alcohol substitution, titration with a few drops of silver nitrate produces a white precipitate of Cl^-, i.e., sodium chloride remains in the interstitial water, thus incomplete replacement of pore water. Fresh water sediments will normally not have the Cl^- ion present, thus the silver nitrate test cannot be used as an indicator for exchange of interstitial water. The replacement step can be hastened by twice daily replacement of the ethanol, and is dependent on the permeability and sample size of the clay. Too much cutting and handling increases the possibility of sample disturbance. A significant advantage when preparing high water content sediment samples (soft or plastic in behavior) is to delay final trimming until after replacement with ethanol or complete dehydration because the clay often becomes more brittle.

Step 3. The absolute ethanol is replaced by amyl acetate, a transition fluid miscible with ethanol and with a lower dielectric constant than ethanol. An ethanol–amyl acetate–liquid CO_2 exchange electrochemically provides an optimal and gentle treatment of the fabric. Replacement of the ethanol with amyl acetate should take approximately the same amount of time as water replacement to ensure complete substitution. Following the replacement of ethanol with amyl acetate, the samples are wrapped individually in wetted lens tissue or placed in a perforated stainless-steel sample holder, and immersed in amyl acetate until the CPD step. The holder consists of eight internal compartments 9 × 5 mm, or configured into four compartments 9 × 10 mm for critical point drying (Fig. 35.2a).

►

Figure 35.1. Flowchart of the procedures for the preparation of sediment samples using SEM and TEM. Steps 1–6 are identical for TEM and SEM and describe dehydration and fluid replacement techniques. Steps 7T–14T describe impregnation, trimming, and ultrathin sectioning. Steps 7S–11S describe fracturing, mounting, and sputter-coating of sample for SEM. Extended from Bennett, 1976.

SAMPLE PREPARATION TECHNIQUES FOR MICROFABRIC ANALYSIS

DEHYDRATION

STEP	TECHNIQUE	INSTRUMENTATION OR DESCRIPTION
1	subsampling of clay specimen and trimming	wire knife
2	replacement of interstitial water with ethyl alcohol	silver nitrate test sealed glass containers
3	replacement of ethyl alcohol with amyl acetate	protected specimen in lens paper or perforated sample holder
4	replacement of amyl acetate with liquid CO_2	critical point apparatus
5	critical point drying with CO_2	critical point apparatus
6	placement and storage of individual specimen in desiccators	samples in vacuum desiccated or sealed jar with desiccant

TEM ONLY

7 T	placement of specimens into individual containers & into vacuum desiccator	plastic cup or Beem capsule protective lens paper removed
8 T	specimens impregnated under vacuum and removed	vacuum desiccator Spurr epoxy resin
9 T	specimens cured at 60° - 70° C	oven
10T	trimming of specimens prior to ultrathin sectioning	glass knife or manually with a razor blade
11T	ultrathin sectioning with microtome	diamond knife microtome
12T	placement of sections on copper grids	copper grid
13T	light carbon sputtering of ultrathin sections	vacuum evaporator
14T	ultrathin sections photomicroscopy	T.E.M.

SEM ONLY

7S	fractured specimen	razor blade
8S	mounted specimen	aluminum stub
9S	electrostatically clean fractured surface	CAB plastic tube sample
10S	sputter coating with palladium/gold wire	vacuum evaporator
11S	Surface feature photomicroscopy	S.E.M.

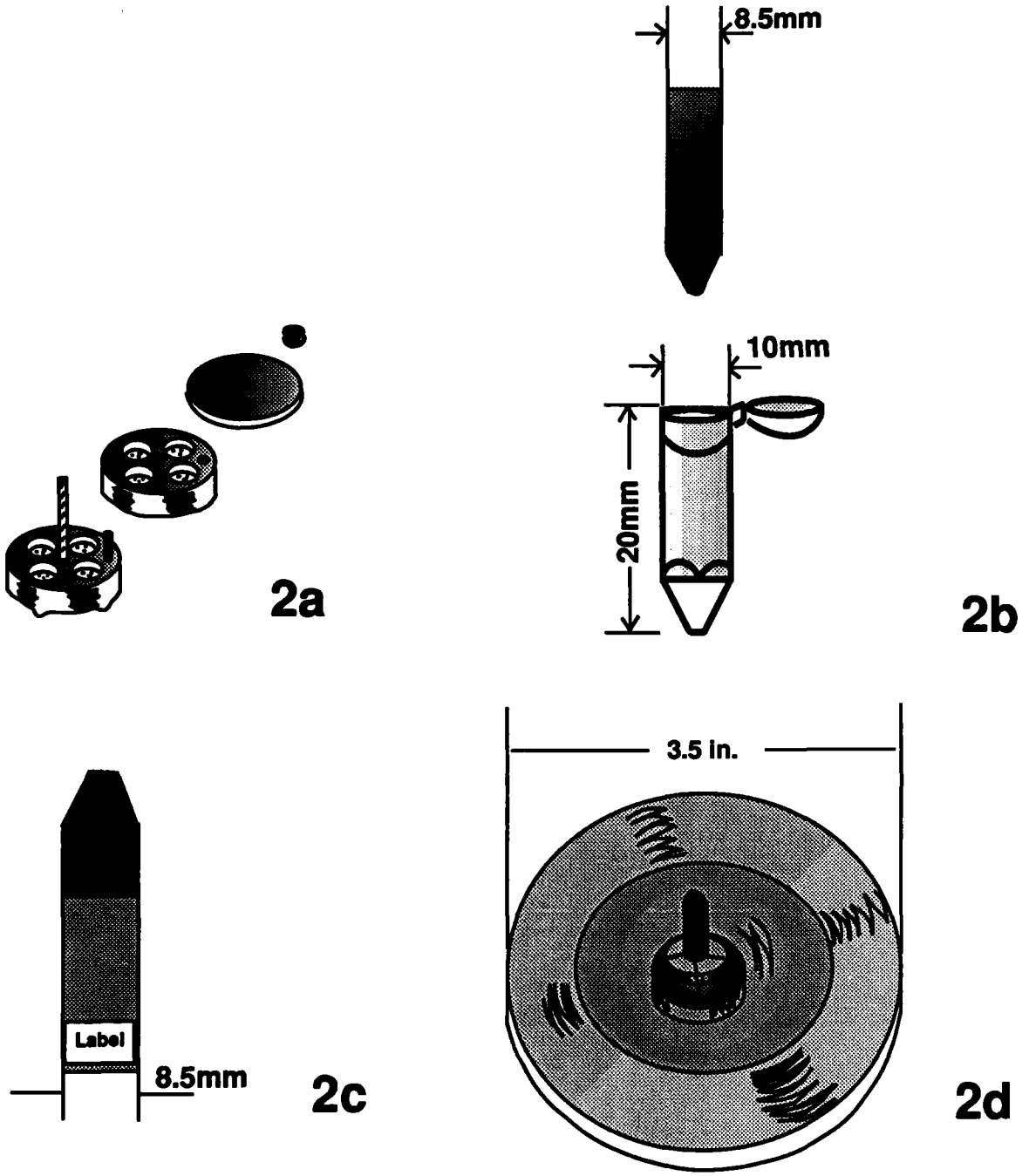

Figure 35.2. (a) Perforated specimen holder capable of containing eight small or four larger clay samples used in the CPD apparatus. (b) BEEM capsule mold for impregnating clay sample into Spurr low viscosity epoxy resin, and the embedded sample. (c) Enlargement of the sample showing embedded label. (d) Machined stainless-steel chuck for holding sample stationary for hand trimming under the binocular microscope.

Steps 4 and 5. Samples are critical point dried in a critical point apparatus using liquid CO_2 as the replacement fluid for the amyl acetate. At the critical temperature and pressure (31.3°C and 72.9 atm), no boundary exists between the liquid and the gas phase, because the density of the gas and liquid phase is identical (Anderson, 1951). Thus the sample can be dried without the deleterious effects of surface tension. Carbon dioxide is preferred because it is easily obtained, inexpensive, and can be incorporated into various types of critical point dryers at a safe and manageable pressure and temperature, for standard labora-

tory procedures. The amyl acetate is rapidly replaced with liquid CO_2 compared to previous transition steps because the liquid CO_2 is slowly purged through the sample chamber. This procedure continuously drives the chemical interface between amyl acetate and liquid CO_2 to "zero" (amyl acetate) by constant replacement with purging fluid. The time required for complete replacement of amyl acetate by liquid CO_2 varies between 20 and 60 min depending on sediment permeability (Bennett et al., 1977).

Step 6. After the samples are dry they are stored in a vacuum desiccator, either individually wrapped in lens paper, or contained in the perforated sample holder. It is critical that the samples remain completely dry for preservation of the structure and for complete resin impregnation.

Extended Procedures for TEM

Step 7T. BEEM capsules (Fig. 35.2b) are oven dried for moisture removal and stored in a desiccator. This is to prevent any moisture adsorption onto the clay surface or resin that may prevent complete polymerization.

Step 8T. The SPURR low-viscosity resin (Spurr, 1969) is mixed according to the manufacturer's hard cure formula consisting of

10.0 g vinyl cyclohexene dioxide (VCD)
26.0 g nonenylsuccinic anhydride (NSA)
4.0 g diglycidyl ether of polypropylene glycol (DER)
0.4 g dimethyl amino ethanol (DMAE) catalyst

Note: The resin should not come in contact with skin or be inhaled as the DMAE catalyst is reported to be a carcinogen. Using dust-free gloves, handle the uncured resin under a fume hood for maximum safety. We experimented with a nontoxic, lower viscosity resin, L.R. White, which required no mixing of components, but unfortunately distortion and warping occurred on curing. Therefore it is useless for fabric studies involving delicate, fine-grain, high porosity, clayey sediments. It could have some potential for embedding less sensitive materials such as well-cemented or shaley fabrics, but it is not recommended for precision studies of clay samples.

Individual samples are placed in BEEM capsules, placed in a tray, and positioned in a vacuum desiccator with proper plumbing to a rotary vacuum pump and to the funnel filled with SPURR (Fig. 35.3). Jim (1985) also advocated the use of SPURR for clay microstructure studies. A curved or bent glass tube with a dropper type end is attached through a rubber stopper and connected to a hose which in turn is connected to the funnel. A clamp on surgical tubing controls the flow of resin. A vacuum is slowly applied to the desiccator to reach optimum vacuum with a standard rotary pump. After about 0.5 hr under vacuum, the resin is slowly released to each BEEM capsule through the glass dropper tube and allowed to run along the side

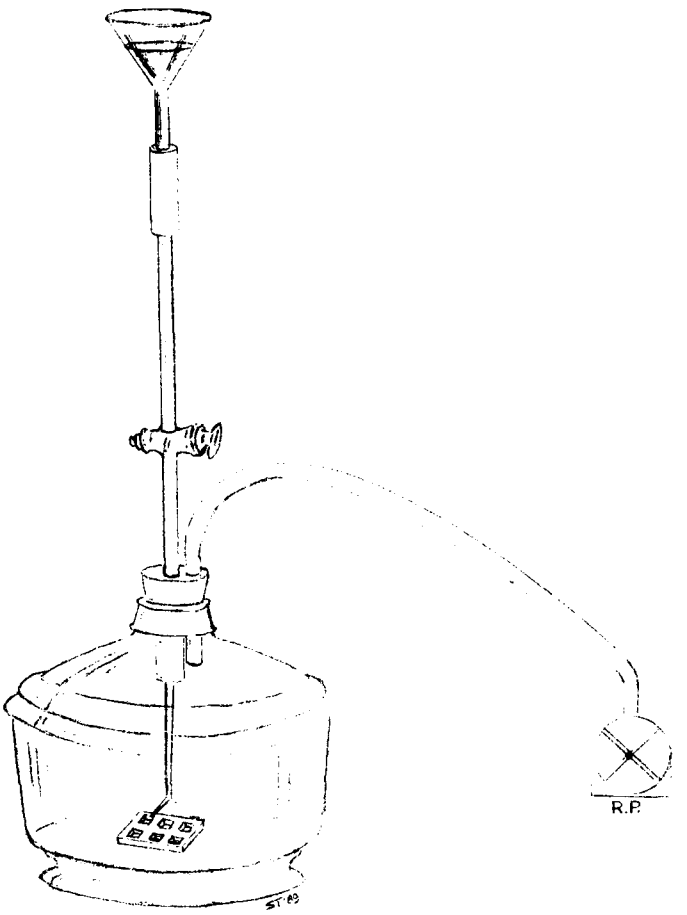

Figure 35.3. Overview of vacuum desiccator assembly for Spurr infiltration of sediment samples under vacuum.

of the BEEM capsule (do not drop resin directly on the sample). The BEEM capsule is filled with resin, the vacuum pump turned off, and the desiccator *very slowly returned* to atmosphere. After impregnation of the resin, a small probe (dental tool or bamboo stick) can be used to carefully adjust the final desired position of the sample and the label inside the capsule. Since SPURR components are toxic, it is safest to carry out all of the above activities in a fume hood with the exhaust fan running.

Step 9T. The samples are placed in a 70°C oven for 24 hr to polymerize the epoxy into a very hard plastic. The best procedure is to vent the oven through a fume hood during curing to avoid obnoxious fumes. The hardness of the resin can be adjusted by changing the relative portions of the four components, (see manufacturer's instructions) but the goal (though difficult to achieve completely) is to reasonably match the hardness of the sample material for consistency during ultrathin sectioning. Many marine clay sediments contain brittle illite, and the hard cure recipe is preferred. Waste resin should be cured and then discarded to avoid health risks. Also cure any of the leftover resin to prevent a sticky lab accident and alleviate any health

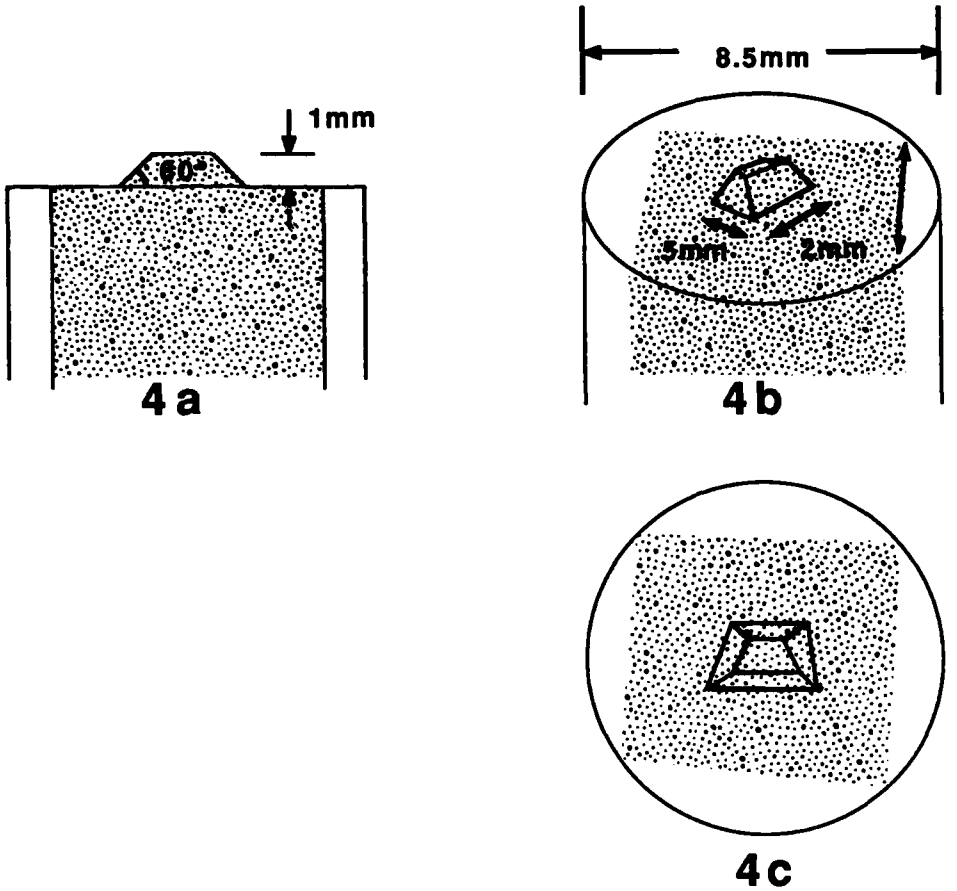

Figure 35.4. Diagram of the tip of the sample showing the geometry achieved by hand sectioning using razor blades. (a) Side view showing the 60° slope of the shoulders providing support during ultrathin sectioning. (b) Trapezoid is carved onto the block face. This shape aids in removal of sections from the knife edge during the follow-through stroke and adhesion of consecutive faces forming a ribbon. (c) Top view.

concerns. Remove cured samples from oven and immediately return to the desiccator to cool. Later remove the cured sample block (Fig. 35.2b,c) from the BEEM capsule using a razor blade. The inability to scratch the epoxy surface with your fingernail indicates a proper hard cure.

Step 10T. The sample is held stationary in a chuck (Fig. 35.2d) and viewed under a binocular dissecting microscope for the trimming steps. Optimal control of trimming the sample prior to ultrathin sectioning is achieved manually using razor blades. A new razor edge should be used for each stroke to prevent scarring and gouging of the block face. A fine file can also be employed usefully for trimming. Starting from the outside of the block, remove the four sides, and slowly work toward the center. As the clay is approached, angle the razor blade to cut about 60° (Fig. 35.4a) forming pyramid-shape sides that provides "shoulders" for stabilization during ultrathin sectioning. As the final size is approached, angle two opposite sides so that the desired final shape of the surface of the face is trapezoidal 0.5 × 1 mm (Fig. 35.4b,c). This shape is ideal for serial section analysis because adjacent edges of the sections adhere to each other and the shape facilitates orientation when viewing a ribbon under low magnification under the TEM. However, if serial sections are not required, a square or rectangle shape is preferable with the sides *not* parallel. This will ensure that the sections will not stick to each other and usually a maximum number of sections can be picked up with one try, since the sections will accumulate near the cutting area of the knife. As an alternate trimming method, a glass knife mounted in a microtome can be used but is more time consuming. The glass edge must be moved and realigned frequently to provide a clean sharp surface.

Step 11T. The trimmed sample block is placed in a microtome and ultrathin sectioned using a diamond knife mounted on a "boat" filled with water (Fig. 35.5a). The microtome incrementally draws the sample across the stationary knife and slices the ultrathin sections. The desired thickness of the ultrathin sections are approximately 800 Å, and appear gold because of their interference colors. The sections adhere to each other forming ribbons of floating trapezoid-shaped samples. The ribbons are then clustered using a single eyelash brush (Fig. 35.5b) (see Hayat and Zirkin, 1973).

Step 12T. A #300 mesh copper grid is held by its edge with forceps and lowered onto the sections, following through into the water, and turned over as it is brought out of the water. As an alternate technique, the grid may be immersed and brought up

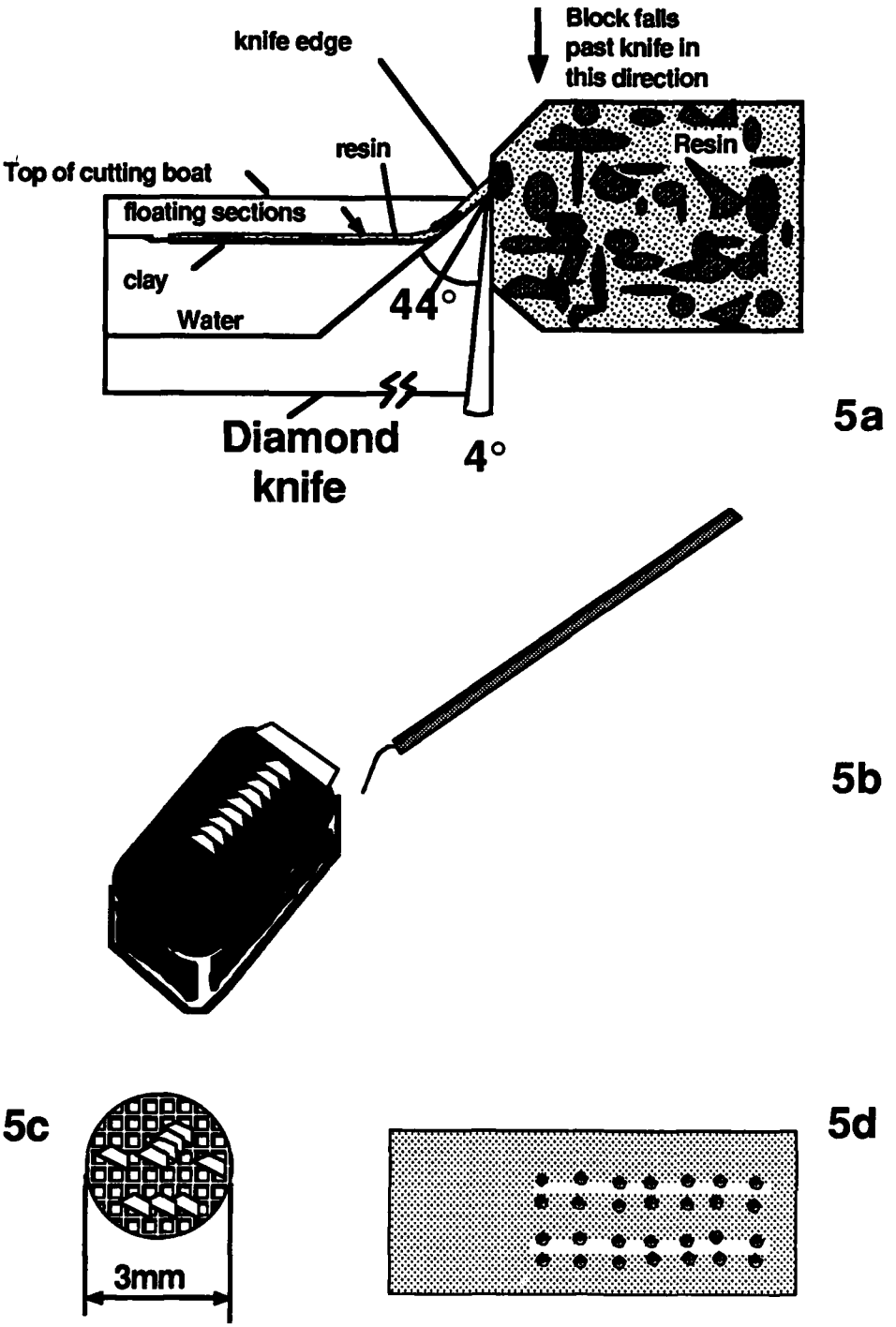

Figure 35.5. Diagram of the diamond knife during ultrathin sectioning of processed, embedded clays. (a) The thin sections float on the water-filled boat (Redrawn after Bryant and Bennett, 1988.) (b) Ribbons can be easily seen through the binocular microscope with proper light and water adjustments. The eyelash brush is used to cluster and manipulate the sections prior to picking up on a copper grid. (c) Enlargement of the copper grid after attachment of the thin sections, by cohesion during wicking onto lens tissue. (d) Standard glass slide showing placement of copper grids onto the edges of thin strips of double stick tape prior to evaporation of carbon for stabilization.

under the sections. The ultrathin sections are now positioned on top of the grid and the water is wicked onto filter paper (below the grid), causing the sections to adhere to the grid (Fig. 35.5c). The grids are stored in numbered grid boxes or clean BEEM capsules and carefully logged. A common but tragic occurrence is spilling, by opening the box too fast or upside down and subsequent mixing of grids.

Note: Grids vary in mesh size, commonly from #100 to #500. For adequate support of the sections and still enough "window" for viewing, a #300 is preferred.

Step 13T. Evaporation of a thin layer of carbon (pale gray as judged by a filter paper reference) unto the thin sections on the grids aids in stabilization by preventing charging of clay particles, etc. under the electron beam in the TEM. Strips of double-stick tape, about 4 mm wide are adhered to a glass slide (Fig. 35.5d). The copper grids are carefully placed face up on the tape so only the outer edge of the grid is adhering. Placing the grids close together allows even distribution of the vaporized carbon. The slide is placed in the vacuum evaporator, and current is generated through sharpened graphite rods resulting in deposition of carbon onto the copper grids. The grids are carefully removed from the glass slide after the chamber is cooled and vented, and stored in the grid box.

Step 14T. The samples are now ready for viewing and photographing in the transmission electron microscope. The photoprocessing commonly uses Kodak sheet film and chemicals with their recommended times. Other commercial sources such as Ilford or Agfa can also be used. Many TEM designs permit the use of 35-mm, 70-mm, as well as $3\frac{1}{4} \times 4$-in. film formats, each with a set of advantages and disadvantages depending on need.

The completed TEM procedure for subsampling and processing the sediment cores through developing and printing of the negatives and prints takes between 20 and 40 hr per sample, but fortunately several samples are processed simultaneously.

Extended Procedures for SEM

Initial preparation of the clay samples for scanning electron microscopy is the same as for TEM through the critical point drying step. The dried samples are stored in a desiccator prior to sputter coating.

Step 7S. The sample must be fractured to reveal a freshly exposed surface. A razor blade is pushed slightly into the sample and twisted causing fracturing of the sample.

Step 8S. The sample is then glued to the surface of an aluminum stub with a drop of carbon conductive paint or a glue, then allowed to thoroughly dry.

Step 9S. Following the procedure of Hulbert and Bennett (1975), a cellulose-acetate-butyrate (CAB) plastic tube rubbed with a lint-free cloth is passed above the clay surface resulting in cleaning with an electrostatic field and freeing the surface of loose particles. This cleans loose particles from the surface and avoids physical contact with the sample surface to be observed with the SEM.

Step 10S. The mounted stub is sputter coated with gold/palladium. The coating should be sufficiently heavy to reduce or prevent charging of the clay surface.

Step 11S. The sample is ready for viewing and photographing in the scanning electron microscope.

The procedures described above are for cohesive sediment samples typically comprised of clay minerals. Often samples predominantly of carbonate minerals are extremely fragile and disintegrate on drying because they lack cohesion. Alternative methods of preserving their structure have been developed and are described below.

Techniques for Carbonate Sediment Preparation for SEM

Often the surface of a sediment sample is too disturbed from the sampling procedure for reliable undisturbed structure studies by SEM. Several methods of exposing new or "fresh" surfaces by various fracturing techniques have been described by Smart and Tovey (1982). In addition, Tovey (1970) and Wong and Tovey (1975) have utilized "peeling" techniques consisting of silver glue, epoxy, plastic, or Sellotape for removal of thin layers of sediment for EM studies.

Scanning electron microscopy of cored, noncohesive carbonate sediment samples is difficult because of their extremely friable nature after critical point drying. Using standard techniques (Fig. 35.1 Steps 7S–11S) for clay samples usually results in disintegration of carbonate fabric under the electron beam or at various stages during preparation. Unstable specimens of any kind create erratic bursts of secondary electrons emitted from the specimen. The effect is known as charging. When charging occurs, the image is obscured on the CRT and photography becomes impossible due to the long photographic time exposures (45 sec to 1.5 min) necessary for low-noise, high-resolution negatives.

Special sample preparation techniques and microscope adjustments are required to preserve the integrity of the microfabric of carbonate samples (first reviewed in Bennett et al., 1990). After critical point drying, oolitic carbonate samples should be stored in a vacuum desiccator to prevent absorbed moisture. Friable samples will often explode when placed into the vacuum chamber if they are not completely free of water. Sample stability is greatly increased by using a low viscosity cyanoacrylate (Superglue) before metal coating. Enough glue should be applied to one side of the sample to penetrate approximately one-third of the sample.

The glue should be allowed to dry completely. Gently break open the sample to expose an undisturbed, glue-free interior surface held together by underlying hardened glue. The specimen should be mounted on a SEM specimen stub with cyanoacrylate and coated with a thick (deposit on sample should have a dark silvery-black color) layer of gold-palladium in the sputter coater. Too thin a coating results in charging, but too thick a layer will obscure structural details. Paint the lower edges of the sample and exposed aluminum stub with a layer of colloidal carbon ("TV TUBE KOAT") to alleviate charging.

In spite of these precautions, the sample remains fragile. Therefore, the SEM settings are important for high-resolution images. The kilovoltage setting should be moderately high (17–20 kV) to compensate for a necessary small probe size setting. Finally, a relatively high (200 μA) emission current is also required to compensate for the smallest possible probe size. A

very small probe size will ensure high-resolution microscopy and at the same time reduce the likelihood of specimen damage or contamination resulting from heating by the scanning beam.

Preparation of Large-Area Ultrathin Films for TEM

Large area ultrathin films provide a substrate and support medium when attached to #300 or #400 copper grids. This technique has applications for clay and mineralogy studies such as selected area diffraction analysis for individual crystal identification, or serial section analysis for pore geometry studies. The various methods described in the literature for the preparation of ultrathin films for transmission electron microscopy (Pease, 1964; Sjostrand, 1967; Hayat, 1970) are all useful, but results are sometimes inconsistent depending on the researcher using the technique. Formvar and Parlodion are popular plastic used for making films. Very large ultrathin films may be prepared easily by applying drops of Parlodion dissolved in amyl acetate to the surface of a bowl of double distilled water. The solution quickly and quite evenly spreads out on the water surface. Rapid evaporation of the solvent leaves behind the pure plastic film that may be affixed to TEM grids in various ways. Unfortunately, ultrathin films produced in this manner are fragile, and can be easily broken mechanically or by heat produced by the electron beam. This problem is especially acute when large area films are required for single hole grids for serial section analysis. For best results, the attached films should be stabilized with a thin layer of carbon from a conventional carbon vacuum evaporator (Fig. 35.1, Step 13T) after the films have been attached to the grids.

Ultrathin films prepared from Formvar plastic are much stronger than Parlodion, and do not require carbon support. Properly prepared, these films are stable under the electron beam for timed exposures of 2 min or longer. This stability is achieved even with very large films stretched over single hole grids used for serial section analysis. However, consistency in preparation of ultrathin Formvar films is difficult. The fundamental problem is that the solvent, commonly ethylene dichloride, used to dissolve the Formvar does not spread evenly on an aqueous surface. The films must be dried onto a solid surface and then released, which can be difficult. Considerable sagging commonly occurs when attaching the film to large hold grids. Since 3-mm copper grids are thin, this sagging will inadvertently allow the film to attach itself to the solid substrate beneath as well as to the grids themselves. This results in destroyed or weakened films caused by the removal of grids from the substrate.

The University of New Orleans Biology laboratory has developed the following procedure that retains some conventional techniques while incorporating new methods. The significant improvements include better release of Formvar films from the glass slides and improved application of the films to the large hole grids. We have realized approximately a 90% success rate using the following techniques:

Figure 35.6. View of setup for attaching Formvar film to large hole grids. Resting palm of hand on edge of staining dish will ensure a steady hand during this process.

1. Rinse and fill a large staining dish (20 cm diameter × 8 cm high) with distilled water.
2. Place two No. 6 rubber stoppers as a base for an aluminum plate (8 cm × 8 cm × 1.5 mm thick) milled with 50 holes (2.8 mm D) plus two additional holes for lifting the slide with microforceps (Fig. 35.6). The holes must be slightly smaller than a standard 3-mm grid to support the entire circumference of the grid (Fig. 35.7).
3. Adjust the water level to approximately 0.5 cm above the aluminum plate and position new, single-slot, oval-shaped grids over the holes in the plate. Be careful to avoid air bubbles while placing the grids on the slide, shiny side up.
4. Wash several smooth glass slides with Comet or some other mildly abrasive detergent.
5. On a wet glass slide place a drop or two of liquid soap (7× cleaning solution works well) on both sides of the glass slide, diluted with a few drops of water.
6. Gently dry the glass slide with paper toweling or Kimwipes until slides look clear when held to a light source.
7. Place glass slides in a 60°C oven to dry for about 15 min. This will prevent excessive holes forming in the film.
8. Pipette several milliliters of Formvar solution (0.5% in ethylene dichloride) and flood approximately 75% of one side of the glass slide.
9. Drain excess liquid immediately from the slide with Whatman filter paper and then tap the end of the slide on the towel to ensure complete drainage of the excess. Air-dry the film for a few minutes.
10. Scrape the sides of the glass slide with a razor blade and make transverse cuts on the film near both ends of the attached film (Fig. 35.8).

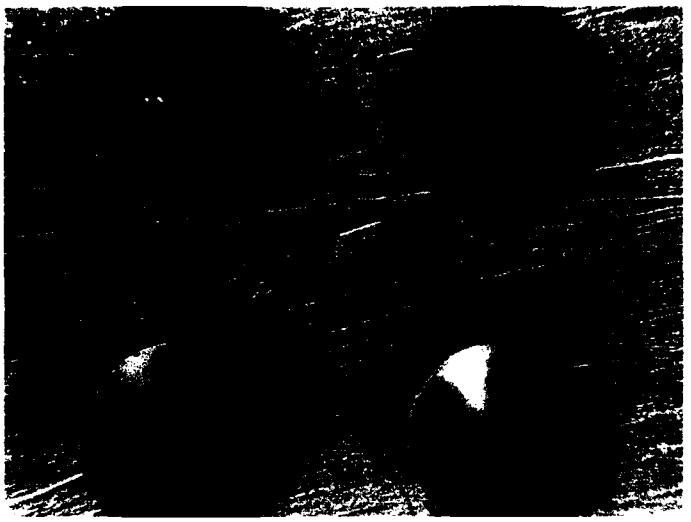

Figure 35.7. Closeup view of 3-mm grids and slightly smaller holes drilled into aluminum support plate. Top two holes have films attached.

11. Position fluorescent light source and vary the angle of the slide with respect to the water surface so that interference colors can be seen.
12. Breathe on the film to create a layer of moisture.
13. Slowly touch the "distal" end of the slide to the water surface watching for the film to attach to the surface meniscus (Fig. 35.9). It may be necessary to assist this process by gently pulling the film with a pair of microforceps. If the film stops coming off at any point, remove the slide from the water, breathe on it again, and resume.
14. Silver to pale gold interference colors represent usable thicknesses that range from 70 to 120 nm (700 to 1200 Å) for routine fabric studies. High-resolution studies would require an excess of 125 kV since it is difficult if not impossible to cut clay microfabric in the "gray" interference color (>50 nm) thickness range.

    Note: If the films appear too thick as judged by blue, green, or pink interference colors, make new films by further diluting the Formvar solution by small increments.
15. Position a suitable floating Formvar film (push gently with microforceps) over the aluminum plate bearing the large hole grids. Carefully lift the aluminum slide straight up with the forceps (Fig. 35.6) and air dry in a standard size petri plate. The grids can be stored on the aluminum plate in a petri plate for later use. Lowering the water level by a siphon device works equally well.

Fixation of Clay–Organic Complexes

An exciting area of investigation is the role of organics in the formation of the microstructure of marine clay sediments. Biogenic components, including fecal pellets and marine snow, are the dominant carriers of organics through the water column. Biological and physical processes can result in resuspension of sediment, also important to sediment microfabric analysis. Complete preservation of *in situ* organics is fundamental for studying the relationships of clay minerals and associated organics. The standard fixation method for most biological specimens for TEM uses glutaraldehyde ($OCHCH_2CH_2CH_2CHO$) followed by osmium tetroxide (OsO_4). An aqueous sodium cacodylate [$Na(CH_3)_2AsO_2 \cdot 3H_2O$] buffer is often used to adjust the concentration of the osmolarity of the tissue. Eight percent glutaraldehyde can be purchased from several vendors (see appendix). The fixative is stored under nitrogen gas in 10 ml vials at 4°C to prevent polymerization of the aldehyde. Working solutions are prepared by making appropriate dilutions with

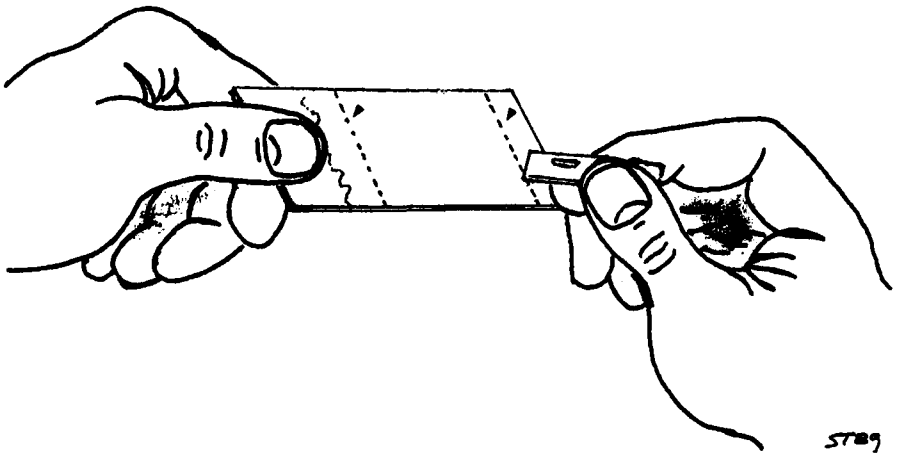

Figure 35.8. Diagrammatic representation of Formvar-coated slide. Positions where cuts in film should be made are indicated with arrows.

Figure 35.9. View of Formvar film separating from the glass slide and attached to the surface meniscus (arrow).

0.2 *M* stock solutions of sodium cacodylate. Sodium cacodylate salt should be stored tightly closed at room temperature because it is hygroscopic. The presence of arsenic prevents mold formation, and allows aqueous solutions to be kept indefinitely at 4°C. Two percent stock solutions of osmium tetroxide should be stored at freezer temperatures to prevent degradation of the solution. The following procedure provides high quality fixation of biological organisms and tissues; currently investigations are underway for preservation of marine clay–organic complexes.

Note: Make sure sample material is completely submerged during the entire procedure. Wheaton snap cap vials (4 × 3 cm) make excellent containers for all steps of this procedure. Be very cautious of the poisonous osmium, and take proper precautions.

1. Break open one 10 ml vial of 8% glutaraldehyde and add it to 10 ml of 0.2 *M* sodium cacodylate buffer. This working solution (4% glutaraldehyde in 0.1 *M* cacodylate buffer) has an osmolarity that is suitable for most organisms.
2. Place sample into solution and fix for 2 hr at room temperature.
3. Place the sample in 0.2 *M* sodium cacodylate for 24 hr to dilute the glutaraldehyde. The sample may be stored this way for up to 1 month at 4°C without damage if fixation needs to be interrupted. For example, because laboratory facilities aboard a research vessel are limited, freshly collected specimens can be fixed in glutaraldehyde then held in cacodylate buffer while being transported to the main laboratory.
4. Remove the cacodylate buffer with a pipette and immediately add OsO_4 (1% in 0.1 *M* cacodylate buffer) to the sample. Fix for 1.5–2.0 hr at 4°C.
5. Postfix with osmium and dehydrate in the normal way as outlined in flow chart starting with the ethanol dehydration step (Fig. 35.1, Step 2).

Future Directions

Major deficiencies exist in our understanding of the microstructure and reactivities of the hydrated states of (1) suspended marine aggregates, such as marine snow and clay floccules and aggregates that may be complexed with organic and inorganic species; (2) weakly cohesive fine-grained sediments such as those that occur at the sea floor or depositional interface; and (3) more cohesive but soft fine-grained sediments.

Scant information exists regarding the influences of biological activity on clays and clay microstructure. Specifically, the effects of biochemical products on clay microfabric development are critical synergistic interactions. Factors include (1) changes in particle-to-particle interactions as a function of clay–organic complexing, (2) chemical alterations of the clay minerals surfaces as a function of changes in the microenvironment caused by microbes and organic material, and (3) effects of organics on the bulk mass physical and mechanical properties of sediments.

Another frontier is the study of the porosities and permeabilities of shales and shaley sediments in terms of (1) the microstructure of the predominant illitic domains and particles, and (2) the diagenetic processes and postdepositional consolidation of muds and fine-grained marine sediments. These questions have major economic significance to the petroleum industry in terms of the large oil-bearing capacities of shales. The limits of current laboratory techniques virtually have been reached and required future investigations include ion milling as an alternative to thin sectioning and pressurized impregnation of resins into the small pore spaces of rocks.

Scientific and technical advances leading toward a functional understanding of the developmental history of the microstructure of suspended aggregates and freshly deposited sediments in terms of the geochemical microenvironment would add new dimensions to our predictive modeling capabilities (Bennett and Hulbert, 1986). These advances will be forthcoming when new technology is available and technology ultimately is pushed to its limit.

An important area of newly developing technology now being applied to clay research is the environmental cell (EC) for TEM. Over the past few years, Fukami, et al. (1987) developed the EC for study and observation of hydrated specimens in the TEM (see Fukami, this volume). Most of the research has been in the study of biological (organic) specimens and chemical reactions that require "natural" hydrated conditions for realistic observations. An EC is now being built at the Naval Oceanographic and Atmospheric Research Laboratory (NOARL) for the study of clay microstructure, clay–organic studies, marine aggregate investigations, and other clay–water chemistry reaction studies (Bennett and Fischer, 1989). The "Wet SEM" (see appendix) is now available to the researcher and awaits its application to clay microstructure, clay petrology, and clay/chemistry interaction studies. Improved methods for sampling marine aggregates and other detritus that preserve the material in the "undisturbed" state are areas of important future research.

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Appendix

Free catalogs and current price lists are available from the distributors listed below. This list includes companies specializing in electron microscope supplies and accessory equipment.

Electron Microscopy Sciences, 321 Morris Rd., Fort Washington, PA 19034
Electroscan, Inc. 100 Rosewood Dr., Danvers, MA 01923 (Wet SEM)
Ernest F. Fullam, Inc., P.O. Box 444, Schenectady, NY 12301
Ladd Research Industries, Inc., P.O. Box 1005, Burlington, VT 05402
Ted Pella Inc., P.O. Box 510, Tustin, CA 92680
SPI Supplies Division of Structure Probe, Inc., P.O. Box 342, West Chester, PA 19380